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PRINCIPAL INVESTIGATOR: Peter Lim, Ph.D.

CONTRACTING ORGANIZATION: SRI International
Menlo Park, CA 94025-3493

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Peter Lim

E-Mail: peter.lim@sri.com

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During the period October 22, 2011 to October 21, 2012, the project personnel continued to perform chemical/physical analyses on bulk pharmaceutical substances and formulated drug products, and to develop dosage formulations of interest to the USAMRMC Drug Development Program for parasitic and infectious diseases, chemical and biological defense, etc. Specific objectives were to design, develop, validate, and apply methods to determine chemical and physical characteristics of the bulk drugs, drug products, to determine their stability under defined conditions, to prepare formulations of bulk drugs for biological testing, and to coordinate ongoing stability studies on an artesunate dosage form with a subcontractor.

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FOREWORD

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_____In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

PI – Signature November 2012

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INTRODUCTION

This annual report for Contract W81XWH-09-C-0022 covers the period from October 22, 2011 – October 21, 2012. The report consists of an overview of the major activities, a listing of the specific tasks performed, reports submitted, and descriptions of special projects performed. The report also includes a listing of personnel receiving pay from this effort, a bibliography of all publications, and meeting abstracts that resulted from this contract during the report period.

This contract is concerned with analytical, characterization, and stability studies of chemicals, drugs, and drug formulations, and with development and manufacture of dosage formulations. The studies are monitored by Mr. William Y. Ellis, the Contracting Officer Representative (COR), Chief, Department of Chemical Information, Division of Experimental Therapeutics, Walter Reed Army Institute of Research (WRAIR).

The overall objective of this project is the operation of an analytical laboratory to determine the identity, purity, strength, quality, physical and chemical properties, and stability of bulk pharmaceutical substances and formulated drug products, and to develop and manufacture, in limited quantities, dosage formulations of interest to the USAMRMC Drug Development Program for parasitic and infectious diseases, chemical and biological defense, anti-viral studies, etc. Specific objectives are to design, develop, validate, and execute methods to determine the following characteristics of candidate bulk pharmaceutical substances and formulated drugs, and to develop and manufacture, in limited quantities, dosage formulations.

- Identity, purity, and strength;
- Stability;
- Other physical and chemical characteristics, including weight variation, content uniformity, and other such compendial requirements;
- Qualitative and quantitative determination of impurities;
- Develop and manufacture, in limited quantities, dosage formulations; and
- Special projects not covered by the above headings.

ANNUAL REPORT (2011-2012)

Overview

During the contract period October 22, 2011 to October 21, 2012, our project work continues to focus on stability determination of the SRI International (SRI)- and the Dalton Pharma Services (Dalton)-manufactured artesunic acid (AS) IV drug products to ensure their suitability for ongoing clinical trial. Units of the SRI Batch No. 14462-16 drug product have been stored/stressed at SRI and at the Army Repository for the first 24 months, and only at the latter site for the remainder of the study. Over all results on SRI Batch No. 14462-16 units indicate chemical and sterility stability for at least 62 months at +5°C and at least 51 months at -20°C. Owing to the dwindling clinical supply of SRI Batch No. 14462-16 and the abundance and availability of clinical supply of Dalton Lot No. 241-1-10-01, the +5°C stability study on Batch No. 14462-16 was concluded. Using the same line of reasoning, the Batch No. 14462-16 stability study at -20°C was also concluded. The second component of the drug product, 0.30M, pH 8.0 sterile sodium phosphate dissolution medium, Afton Lot No. 14290-15, has also shown chemical and sterility stability for at least five years, when stored at 25°C.

Units of the Dalton Lot No. 241-1-10-01 AS IV drug product have been stored/stressed at Dalton for 24 months as a part of the manufacturing/stability determination of the drug product. Overall chemical and sterility results indicate stability for at least 24 months when stored at +5°C and at 25°C. Results on units stressed at 30°C for 12 months indicate continuing sterility, but a slight loss of potency, but still within label value; moreover, quality of the drug remained high. Although the drug potency was still within label value after being stressed at 40°C for 9 months, trace signs of decomposition have begun to appear. After 12 months at 40°C, product potency and quality no longer meet label specifications. All test units maintained sterility integrity. The second component of the drug product, the 0.30M, pH 8.0 sterile sodium phosphate dissolution medium, has also shown chemical and sterility stability for at least two years, when stored at 25°C.

Although the 12-months accelerated and the 24-months shelf-life stability studies on Dalton Lot No. 241-1-10-01 AS IV drug product are completed, the shelf-life stability study needs to continue as long as the clinical supply is in use. Arrangements have been made between SRI and Dalton that AS units stored at +5°C for 36 months at an Army storage facility will be pulled and sent to Dalton for assay. Similarly, phosphate units stored at 25°C for 36 months also will be pulled and sent to Dalton for assay. SRI is to provide the needed reference standards and to monitor the continuing study. When reports from Dalton are reviewed and approved, they will be forwarded to the COR. Identical arrangements for a 48-months pull/assay have been made.

A second major task on which much time/effort were spent during the contract period concerns characterization and stability of 17-α-ethynlestradiol-3-sulfate, sodium. This task will be described under special projects.

The identification and assay of bulk drug substances and dosage formulations continued throughout the contract period.

Specific Tasks Performed and Reports Submitted

During the contract period October 22, 2011 to October 21, 2012, the following tasks were performed and the reports submitted to the COR.

1. WR252425;BU20975 Lot No. 705744, characterization of Glucantime Antimoniate de Meglumina, SRI Report No. 1280.
2. WR256283;BR29487, chemical and sterility/endotoxins stability of artesunic acid clinical dosage form, SRI Batch No. 14462 stored 62 months at 5°C, SRI Report No. 1278.
3. WR256283;BR29487, A summary report detailing the development and manufacture of the artesunic acid IV drug product, 110mg/vial, SRI Batch No. 14462-16 and of the associated sodium phosphate dissolution medium, SRI Report No. 1280. Also described in the same report was the development and manufacture of SRI Batch No. 14462-16 Placebo.
4. WR256283;BR29487, A summary report detailing the shelf-life and accelerated stability studies on the artesunic acid IV drug product, 110mg/vial, SRI Batch No. 14462-16 and on the associated sodium phosphate dissolution medium, SRI Report No. 1284.
5. WR299958;BU57682, characterization of decoquinate, SRI Report No.1279.
6. WR621305, characterization of a sample of 17-α-ethynylestradiol-3-sulfate, sodium, SRI Report No. 1281.
7. WR261361, Lot No. STK 001-41, characterization of 17-α-ethynylestradiol-3-sulfate, sodium, SRI Report No.1283.
8. WR261361, Lot No. STK 001-41, updated summary on 17-α-ethynylestradiol-3-sulfate, sodium bulk stability at 5°C and at 25°C, and as a 1% solution in water at 25°C, SRI Report No. 1285.
9. WR261361;BU61006, Lot No. STK1-83, characterization and stability studies on 17-α-ethynylestradiol-3-sulfate, sodium, SRI Report No. 1286.
10. WR773633;BM18591R, preparation of 1% gentamicin base in the TEVA15% Paromomycin/0.5% Gentamicin Placebo Cream formulation, SRI Report No. 1288.

Special Projects

Among the special projects carried out during this report period, development of a lyophilized artesunic acid IV drug product consumed the most time/effort.

Although two batches of cGMP artesunic acid IV drug product have been manufactured, the active pharmaceutical ingredient (API) in both was sterilized by treatment with ethylene oxide, a process that's commonly used for sterilization of prosthetics but uncommon for pharmaceuticals owing to major drawbacks. Two main ones are: 1) ethylene oxide is a known carcinogen and its hydrolytic product, ethylene glycol, is poisonous; and 2) the process does not lend itself to large-scale operation. For these and other reasons, an improved formulation of an IV artesunic acid is continually sought. In our initial development for such a product, the use of ethylene oxide for sterilizing artesunic acid was probably a last resort because more-commonly employed methods failed. The ideal method of producing an IV product is by lyophilization of a frozen aqueous solution of the API. Our early attempts to lyophilize frozen solutions of sodium artesunate did not yield a stable product, chiefly because an anhydrous cake was unobtainable and the residual water caused API hydrolysis to dihydroartemisinin, which owing to its very limited water solubility is unacceptable as an IV product. Early attempts to prepare a product via non-aqueous lyophilization also failed. In the current quarter, we have proved in principle that a non-aqueous lyo product of artesunic acid is possible, and much of our effort/time is being devoted to this task. A preliminary report on development has been sent to the COR on October 25, 2012.

Another special project concerned characterization of a crystalline material isolated from spent artesunic acid solutions in phosphate buffer collected from earlier assays of units of SRI Batch# 14462-16. The gathered data on these crystals point to deoxyartemisinin, which is a known compound but has not been reported to be found in such solutions. Comparative data with those obtained from a deoxyartemisinin reference standard have verified the identity of our material. We are in the process of writing a paper on the isolation, characterization, and a possible mechanism from which the deoxyartemisin was formed.

Publications and Presentation

No publications resulted from investigations conducted during the report period.

A presentation, Analytical Studies on 17-α-Ethnylestradiol-3-sulfate, Sodium, was made at a SBL meeting in Washington, D.C. on March 12, 2012.

A draft copy of a publication entitled *"Chemical Characterization of Sodium Stibogluconate (Pentostam) in Anti-leishmanial Agent Solutions"* has been written and a publication on *"Formation of Deoxyartemisinin from Artesunic Acid in Solutions of Sodium Phosphate"* is in preparation.

Awards

No awards were received during the report period.

PERSONNEL

A listing of personnel who received major contract support during the report period is as follows:

- Peter Lim, P.I.
- Ronald Spanggord, Assistant P.I.
- Jennifer Wang, Chemist

A listing of subcontractors who received major contract support during the report period is as follows:

- Dalton Pharma Services

SUMMARY/CONCLUSIONS

The five-year shelf-life stability study on units of artesunic acid IV drug product, SRI Batch No. 14462-16 and Afton Batch No. 14290-15B, manufactured in 2004, has been completed. Results indicate chemical and sterility stability for at least 5 year. Owing to the near depletion of the clinical supply of this drug product and the availability of its replacement, stability study on the 2004 drug product is discontinued.

Shelf-life stability study on bulk artesunic acid is past six years and results indicate continuing chemical stability.

The two-year stability studies on Dalton Lot No. 241-1-10-01 and the Afton Lot No. 1023-09, replacements for the 2004-manufactured artesunic acid IV drug product, have been completed. The results indicate chemical and sterility stability for at least two years. The shelf-life stability study on these units is continuing; arrangements for the three- and four-year pull/assay have been scheduled.

The current method of manufacturing the artesunic acid IV drug product has at least two disadvantages: sterilization by ethylene oxide, and dry filling of the individual units. A successful lyophilization would overcome both drawbacks. We have successfully demonstrated in principle a lyo artesunic acid product and are continuing to refine the process and study the product.

Characterization and stability studies on samples of 17-α-ethynlestradiol-3-sulfate sodium represented a major project during the contract year. Much of the effort/time was devoted to determining detrimental impurities that caused instability of the material.

The project team continues to provide solutions to the Army's analytical problems.

Respectfully Submitted:

Peter Lim, Principal Investigator
Phone: (650) 859-3029
Fax: (650) 859-4321
E-Mail: peter.lim@sri.com